Phenol-Chloroform DNA isolation for environmental DNA (eDNA)

Spangler MA, Lopez JA

Abstract

This protocol for isolating environmental DNA from filter membranes is modified from Renshaw et al. 2015.

The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol–chloroform–isoamyl alcohol DNA extraction.

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Protocol

Filter Membrane

Step 1.

Shred 1/2 filter membrane using a sterilized blade and forceps.

Add shredded membrane to 2 mL sample tube.

Store remaining membrane for future use.

DNA Lysis Buffer

Step 2.

Add 800 µL Buffer ATL (Qiagen) to 2 mL sample tube containing shredded filter membrane.

AMOUNT
800 µl Additional info:

REAGENTS
Buffer ATL 19076 by Qiagen

Lysis Incubation

Step 3.

Incubate sample tubes at 65°C for 10-15 minutes.

Vortex occassionally throught incubation period.

DURATION
00:15:00

Phenol-Chloroform-Isoamyl Alcohol

Step 4.

Add 800 µL PCI (one phase 25:24:1, Amresco) to sample tubees.

Vortex briefly to mix layers.

Centrifuge for 5 minutes at 15,000 g.

REAGENTS
Phenol/Chloroform/IAA K169 by Amresco

DURATION
00:05:00

Chloroform-Isoamyl Alcohol

Step 5.

Transfer 600 µL of the aqueous DNA layer (top) to a new 1.5 mL sample tube.

Add 600 µL CI (24:1, Amresco).

Vortex briefly to mix layers.

Centrifuge for 5 minutes at 15,000 g.

AMOUNT
600 µl Additional info:

REAGENTS
Chloroform/IAA X205 by Amresco

DURATION
00:05:00

DNPrecipitation

Step 6.

Transfer 400 µL of the aqueous DNA layer to a new 1.5 mL sample tube.

Add 1 mL 100% ice cold ethanol.

Add 16 µL 5M NaCl.

Incubate overnight at -20°C.

AMOUNT
400 µl Additional info:

DURATION
12:00:00

DNA Extraction

Step 7.

Centrifuge sample tubes for 10 minutes at 15,000 g.

Decant and dispose of liquid.

DURATION
00:10:00

Ethanol Wash

Step 8.

Add 100 µL 70% room temperature ethanol to DNA pellet.

Vortex briefly.

Centrifuge for 2 minutes at 15,000 g.

Repeat for samples with high likelihood of PCR inhibition (discolored, gelatinous pellets or samples collected from wetlands with high tannins or humic acids).

AMOUNT
1 µl Additional info:
DURATION
00:02:00

DNA Isolation

Step 9.

Decant and dispose of liquid.

Dry samples in a vacuum heater for 10-15 minutes.

Continue to air dry until no visible liquid remains.

DURATION
00:15:00

DNA Preservation

Step 10.

Add 100 µL 1x TE Buffer, low EDTA.

Incubate at room temperature overnight.

AMOUNT
100 µl Additional info:
REAGENTS
TE Buffer by Contributed by users
DURATION
12:00:00