Simple DNA Extraction for Phytoplankton Using Chelex 100

Version 2

G Jason Smith

Abstract

This is a modification of the origianl Chelex 100 extraction described by Walsh, Metzger and Higuchi (1991 BioTechniques 10(4):506). Adding PVPP facilitates working with environmental samples as well as pure cultures hi in phenolics or other contaminants.

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Protocol

REAGENT PREPARATION

Step 1.

Add 5.0 g Chelex 100 to a clean 50 mL Falcon Tube.

REAGENTS
Chelex 100 C7901-100G by Sigma Aldrich

REAGENT PREPARATION

Step 2.

Add 0.5 g Polyvinylpolypyrrolidone to the same 50 mL Falcon Tube.

REAGENTS
Polyvinylpolypyrrolidone 77627-25G by Sigma Aldrich

REAGENT PREPARATION

Step 3.

Resuspend in 50 mL nuclease free H_2O by vortexing to hydrate.

REAGENTS
Ultrapure Distilled, Nuclease Free Water by Contributed by users

REAGENT PREPARATION

Step 4.

Wash sample and collect suspension by centrifugation at 3000 xg, 10 min.

DURATION
00:10:00

REAGENT PREPARATION

Step 5.

Decant supernatant and repeat nuclease free H_2O wash.

REAGENTS
Ultrapure Distilled, Nuclease Free Water by Contributed by users

DURATION
00:10:00

REAGENT PREPARATION

Step 6.

Decant supernatant and resuspend pellet in 50 mL 0.1X TE buffer, pH = 8. Vortex sample.

REAGENTS
TE Buffer by Contributed by users

REAGENT PREPARATION

Step 7.

Incubate sample with mixing for 10 min.

DURATION
00:01:00

ANNOTATIONS
G Jason Smith 24 May 2017

Lay sealed Falcon tube on its side on shaker table, mix at ca 30 rpm for 10 min

REAGENT PREPARATION

Step 8.

Centrifuge to collect suspension at 3000 xg, 10 min.

DURATION
00:01:00

REAGENT PREPARATION

Step 9.

Decant, and resuspend pellet in 50 mL 0.1X TE buffer, pH=8.

REAGENTS
TE Buffer by Contributed by users

DNA EXTRACTION

Step 10.

Filter samples using 25 mm polycarbonate filters.

DNA EXTRACTION

Step 11.

Aliquot 300 µL of mixed suspension into a 1.5 mL tube.

NOTES

Alyssa Alsante 24 May 2017

Be sure to completely resuspend extraction buffer prior to aliquoting.

For cell pellets--add 300 µL Chelex suspension directly to pellet and proceed as above.

Volume of Chelex can be adjusted for biomass of samples, but 300 µL is a good starting point.

DNA EXTRACTION

Step 12.

Add filter into extraction tube, seal and vortex to resuspend cell sample.

NOTES

Alyssa Alsante 24 May 2017

Do not fold the filter.

DNA EXTRACTION

Step 13.

Heat suspended sample at 65°C, 5 min.

DURATION

00:05:00

DNA EXTRACTION

Step 14.

Vortex and centrifuge at 10,000 xg, 3 min to sediment particles.

DURATION

00:03:00

DNA EXTRACTION

Step 15.

Generally, dilute DNA extract supernatant 1:10 with TE buffer at pH=8 for PCR assays.

REAGENTS

- TE Buffer by Contributed by users

DNA EXTRACTION

Step 16.

DNA concentration and quality can be increased using the Zymo Genomic DNA Clean & Concentrator-10 spin columns following the manufactures protocol.

REAGENTS

Genomic DNA Clean & Concentrator-10 D4011 by Zymo Research