Phenol Chloroform DNA extraction from polycarbonate filters

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Abstract

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Before start

-Sterile polypropylene tubes (15 or 50 ml); sterile Oak Ridge-tubes

-Steel forceps

-Scissors

-glass rod with rounded end

-95% Ethanol for sterilization

-Sterile lysis buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH9)

-Stock solutions

-lysozyme (100 mg/ml)

-proteinase K (20 mg/ml)

-10% SDS

-Incubator/water bath for +37ºC and +50ºC incubations

-Glass pipettes (10 ml)+ rubber bulb

-Saturated phenol, pH 8

-Chloroform-isoamyl alcohol (24:1)

-Absolute and 70% ethanol

Protocol

Step 1.

If not using a whole filter, aseptically cut out a piece (e.g. a 1/2) of a filter.

NOTES
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For aseptic technique, dip steel forceps and scissor into 95% ethanol, then flame forceps. Repeat twice.

Step 2.

Put filter into sterile polypropylene tube.

Step 3.

Place tube on ice.

Step 4.

Add lysis buffer into tubes.

NOTES
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See "Before you start" on options for a lysis buffer.

Step 5.

Massage the filter with a flame-sterilized glass rod to suspend all the cells from the filter.

Step 6.

Add lysozyme from a 100 mg/mL stock to a final concentration of 1 mg/mL.

Step 7.

Incubate 20 minutes at 37ºC

DURATION
00:20:00

NOTES
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Ideally on a shaker, but a water bath is ok.

Step 8.

Add proteinase K (20 mg/ml stock solution). Final concentration of 50 µg/ml.

Step 9.

Add 10% SDS, final concentration 0.5%.

Step 10.

Incubate 2 hours at 50ºC.

DURATION
02:00:00

NOTES
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Ideally on a shaker, but a water bath is ok.

Phenol chloroform extraction

Step 11.

Do all of the following in the fume hood.

Phenol chloroform extraction

Step 12.

Add saturated phenol into tubes, 1/2 of the total aqueous volume.

NOTES

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Saturated phenol can be purchased, rather than made, if preferred.

Phenol chloroform extraction

Step 13.

Cap the tubes tightly, shake the contents well. Allow it to sit for 5 minutes.

DURATION

00:05:00

Phenol chloroform extraction

Step 14.

Same as phenol, add equal volume of chloroform isoamyl alcohol (2.5 mL) and repeat step 2. On a tabletop centrifuge, run for 1 minute on maximum speed.

DURATION

00:01:00

Phenol chloroform extraction

Step 15.

Transfer the aqueous (top) layter into sterile Oak Ridge-tubes.

NOTES

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Oak Ridge tubes.

Ethanol Precipitation of DNA

Step 16.

Add 2x the aqueous volume of ice cold absolute ethanol into tubes.

Ethanol Precipitation of DNA

Step 17.

Add 0.1x the total volume of 10 M ammonium acetate.

Ethanol Precipitation of DNA

Step 18.

Mix the contents and place the tubes in -20ºC freezer for at least 1 hour, or overnight.

DURATION
00:01:00

Ethanol Precipitation of DNA

Step 19.

Spin the tubes at 10,000 rpm for 35 minutes.

DURATION
00:35:00

Ethanol Precipitation of DNA

Step 20.

Carefully remove the supernatant.

Ethanol Precipitation of DNA

Step 21.

Carefully wash the DNA pellet with 1 mL of ice-cold 70% ethanol.

Ethanol Precipitation of DNA

Step 22.

Remove the 70% ethanol and place the tubes in sterile hood and let the DNA pellets dry for 2-3 hours, until completely dry.

DURATION
00:03:00

Ethanol Precipitation of DNA

Step 23.

Resuspend the DNA into 200 µL of sterile TE buffer. (pH 8)

Ethanol Precipitation of DNA

Step 24.

Measure the DNA concentration using the spectrophotometer and/or fluorometer.

LINK:
https://www.protocols.io/view/nanodrop-spectrophotometer-nd-1000-for-nucleic-aci-id2ca8e

Ethanol Precipitation of DNA

Step 25.

Store DNA in a -20ºC freezer.