Labyrinthulomycete DNA extraction protocol

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Abstract

Modified from Lippmeier et al. 2009

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Protocol

Step 1.

Prepare extraction buffer:

20 mM Tris-HCl pH 8.0

50 mM NaCl

10 mM EDTA

0.5% SDS

Also need stocks of

proteinase K (10 mg per ml is handy)

RNAse A (25 mg per ml is handy)

phenol:chloroform:isoamyl alcohol (25:24:1)

chloroform:isoamyl alcohol (24:1)

3M NaOAc (sodium acetate)

100% ethanol

70% ethanol

NOTES
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This protocol has worked well for Aurantiochytrium limacinum ATCC1381 and Aplanochytrium

kerguelense PBS07, meaning that it produced clean spools of genomic DNA.

For Aurantiochytrium, a 50 ml culture in medium 790BY+, 2 or 3 days old, yields ~350-400 mg wet biomass and ~200-300 ug DNA.

This protocol has never worked as well for Schizochytrium aggregatum ATCC28209; the DNA comes out more fragmented and in a background of a small precipitate and had to be collected by centrifugation rather than spooling. (sometimes this happens with the other strains, too) I was able to a cleaner prep by redissolving the initial pellet and performing a LiCl precipitation (final concentration 2M LiCl, incubated at -20C for 1 hour, then centrifuged at 16,000 xg and 4C for 20 minutes) then adding 1 volume isopropanol to the supernatant, which produced a nice spool of DNA (but get it out quickly).

Step 2.

Resuspend 100 mg wet weight fresh cell pellet in 1 ml extraction buffer plus 100 ug per ml proteinase K

Incubate at 45 minutes at 50C

(can spin down cells from liquid culture, or washed from agar plate, at 4000xg (in C0650 rotor), room temp, 10 minutes; wash in 50% artifical seawater then ddwater before a final spin and weighing pellet)

Step 3.

Add 100 ug per ml RNAse A

Incubate for 15 minutes at 37C

Step 4.

Extract with an equal volume of phenol:chloroform:isoamyl alcohol

Mixing gently for 30 minutes at room temperature

Gently centrifuge (8,000 x g, 10 minutes) to separate phases

Transfer aqueous phase to a fresh tube

REPEAT

Step 5.

Extract with an equal volume of chloroform:isoamyl alcohol

Mixing gently for 5 minutes at room temperature

Gently centrifuge (8,000 x g, 10 minutes) to separate phases if needed

Transfer aqueous phase to a fresh tube

REPEAT

Step 6.

Add 0.1 volume 3 M NaOAc

Add 2 volumes ice-cold 100% ethanol

DNA should precipitate as a fluffy ball; collect by spooling onto a glass rod

(a Pasteur pipette with the open end melted closed works well)

Wash by dipping in 70% ethanol

Air dry

Dissolve in 100-350 microliters water or TE (pH 8.0)

(depending how much biomass went in)

Warnings

Be careful with phenol:chloroform and chloroform; wear appropriate PPE.