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Abstract

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Filamentous fungi are notorious for their low DNA and high RNA contents as well as their rigid cell walls. I have been surveying genetic differences such as RFLPs in isolates of obligately anaerobic rumen fungi of the class Chitridiomycetes. These organisms present the added complications of slow growth in unconventional culture conditions, hence material is limited, as well as exceptionally low in DNA content by weight. In some instances as much as 35% of the dry weight is a glycogen-like storage polysaccharide (Concanavalin A-reactive) that copurifies with DNA through many purification steps including CsCl density gradient centrifugation. This and other acidic polysaccharide contaminants severely inhibit restriction enzymes and DNA ligase.

I describe a rapid procedure for isolating high MW genomic DNA from fungi (or any organism for that matter) where any of the above problems are encountered. The srparation of DNA from contaminants is based on the two phase technique of Kirby (1956, Biochem. J. 64:405-408) combined with polyethylene glycol precipitation of DNA from the phosphate-rich organic phase. The protocol is applied to 50 mg dry weight of starting material but can probably be adapted to larger amounts.

1. Lyophilized mycelium is ground to a powder in a mortar and pestle. 50 mg is placed in a microcentrifuge tube.
2. Material is suspended in 500 ul of 0.2 M Tris, 0.25 M NaCl, 0.025 M EDTA (pH 8.5) containing 0.5% SDS.
3. Add 10 ul of protease K (20 mg/ml), incubate at 65° for 30 min. (Note 1).
4. Add 500 ul of chloroform isoamyl alcohol (24:1), mix well and centrifuge 5 min. (Note 2).
5. Transfer the upper phase to a clean tube and add 0.7 vols. (350 ul) of isopropanol at room temperature, mix, leave 5 min.
6. Centrifuge the sample for 5 min, drain tube and dissolve the pellet in 200 ul sterile TE [10mM Tris, 1 mM EDTA (DH 8.0)]. Warming mav help. (Note 3).
7. Add 200 ul of cold 2.5 M potassium phosphate (pH 8.0) then 200 ul of cold 2-methoxyethanol (ethylene glycol monomethyl ether), mix well and centrifuge for ca. 2 min.
8. The DNA in the upper (organic) layer is transferred to a clean tube (~500 ul) avoiding the interface material. Add an equal volume of HOH and ca. 330 ul of 30% polyethylene glycol 6000 in 1.5 M NaCl. Mix well and leave on ice 15 min. (Note 4)
9. Centrifuge the tube for 5 min (12,000 x g) and remove the supernatant. Centrifuge again briefly to ensure that all of the viscous solution is at the bottom of the tube and remove it.
10. Dissolve the pellet in 200 ul TE (warm if necessary) then add 200 ul 5 M ammonium acetate and 1 ml ethanol at room temperature. After 10 min centrifuge the tube for ca. 10 min, drain and rinse the pellet with 70% ethanol. Dry the pellet briefly under vacuum and dissolve it in an appropriate volume of TE (50 ul). 5 ul of this solution is sufficient to check size and recovery on an agarose gel.

Notes: (1) Protease K treatment substantially increased the yield of DNA in several trials. (2) The use of phenol or extended high speed centrifugation at this step (see Raeder and Broda, Letts. Appl. Microbiol., 1985, 1:17-20) is unnecessary. (3) RNAse treatment can be included at this stage but generally the RNA content of the final product is very low without it. (4) The final concentrations of PEG and NaCl are not critical. The object is to achieve 6-8% PEG in high salt while diluting low molecular weight contaminants including RNA, as much as possible (having regard to the nominal capacity of a 1.5 ml microcentrifuge tube). DNA can be precipitated from the high phosphate buffer with cetyltrimethylammonium bromide (Bellamy and Ralph, 1968, Meth. Enzymol. 12:156-160) or cetylpyridinium bromide (Geck and Nasz, 1983, Anal. Biochem. 135:264-268) but the recovered DNA does not digest as well as when PEG is used and the RNA content is higher.

The yield is usually 10-12 ug of high MW DNA (>30 kb by agarose gel electrophoresis) that is readily restriction digested or ligated in small volumes. I should emphasize that the method described here, unlike many others I have tried with this group of fungi, results in DNA of substantially improved yield and degree of purity yet is simple and rapid enough to permit joint processing of several samples on a small scale. The occasional sample with persistent resistance to restriction digestion can be further improved by spermine precipitation (Hoopes and McClure, 1981, Nucl. Acids Res. 9:5493-5504; Pingoud et al., 1984, Biochem. 23:5697-5703). Ms. D. Copelin was of great assistance in the development of this method. - - - CSIRO, Division of Animal Production P.O. Box 239, Blacktown, NSW 2148, Australia.