How to isolate DNA; Large Scale.

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Background

It is often necessary to isolate genomic DNA from Neurospora strains, to check for particular single nucleotide polymorphisms or to screen transformants for inserted sequences. Both of these can be done using PCR and so require only a small amount of relatively pure DNA. However, if several Southern blots must be done, or a genomic library made of a strain that differs from that sequenced by the Genome project, it may be desirable to isolate a large quantity of good quality DNA

Procedure

Neurospora genomic DNA is prepared using an adaptation of the method of Schectman (1986). A conidial suspension of the desired strain is inoculated into 50 ml of liquid Vogel's medium (supplemented as necessary) in a 250 ml flask and shaken at 25°C for 2-3 days, until the culture is saturated. If the culture is Cot^+, growth can be at 34°C for a shorter time. We usually keep the cultures in the dark, to discourage conidiation, and give them a good swirl by hand once to twice a day to prevent mycelium growing up the sides of the flask.

The mycelium is harvested by pouring the culture through a funnel lined with two layers of sterile muslin, or passing through a filter paper in a Buchner funnel. Excess liquid is removed by pressing mycelium between layers of paper towel. At his point the mycelial mat can be stored at -70ºC. Where possible, the mycelium should be freeze-dried for 24-36 hours, after which it can be stored at -20ºC until needed. If using mycelium that had not been freeze-dried, of course you need to use more. The procedure is equally successful, although more difficult, as wet mycelium must be ground under liquid nitrogen.

1g of dry mycelium is ground to a fine powder in a pestle and mortar. The powder is added slowly (to minimise clumping) to 10 ml of Neurospora SDS buffer (0.15M NaCl, 0.1M EDTA, 2% SDS, pH 9.5) in a 125 ml flask. Add 0.5 ml 2mg/ml proteinase K solution and incubate at 37°C for up to 24 hrs with gentle agitation.

10 ml sterile water is added to the slurry and cellular debris is removed by centrifugation at 10,000 rpm/10min/4ºC. The supernatant is extracted 3-5 times with phenol saturated with Tris-HCl (pH 8.0) and once with water-saturated chloroform.

The DNA is precipitated by the addition of 2.5 volumes of absolute ethanol and removed with a sterile glass hook to an Eppendorf tube. If this doesn't work for you, you can centrifuge briefly and remove the supernatant. After air-drying briefly, add 1ml sterile 10mM Tris-EDTA, pH 8.0 and 30µg. Do not use any vigorous means of resuspending the DNA, as it will shear easily. After incubation at 37ºC for several hours (or overnight) with occasional mixing by inversion, the DNA should dissolve by itself. To remove the RNAse, add 80µg of proteinase K, and continue incubation at 37°C for a further 2 hours.

The opalescent liquid is then extracted 3 times with phenol/Tris and twice with chloroform/H_2O. Precipitate DNA with 2.5 volumes of ethanol (brief centrifugation only required), wash with 70% ethanol, dry briefly and resuspend in up to 2 ml 1xTE.

This method has yielded 1-2 ml of genomic DNA sufficiently concentrated that 2µl are sufficient for a non-radioactive blot. DNA I have made as long ago as 1992 is still in very good condition after extended storage at 4ºC.