Feher, Zs. and M. Schablik

Fast preparation of Neurospora DNA for Southern analysis.

We propose two methods for preparation of DNA from small amounts of Neurospora crassa mycelium. Both methods were described for yeast (Struhl et al 1979 Proc. Nat. Acad. Sci. USA 76: 1035-1039, Kingsman et at. 1979 Gene 7: 141-151) and were adapted for Neurospora with minor modifications.

Mycelia for DNA preparation were grown either on cellophane-agar plates (Schablik et al. this issue) or in 100 ml liquid overnight (16-18 hours old) cultures.

Protoplast formation was carried out in 1 M sorbitol, 10 mM MOPS (pH 5.8) containing 1% Helicase L'lndustrie Biologique Francaise) and 1% Chitinase (from Serratia marcescens, SERVA) with gentle shaking at 27.29°C for 2 hours (Schablik et al. this issue). Spheroplasts or osmotically unstable hyphal fragments can be obtained with the same treatment from submerged culture, as well. The protoplasts (or spheroplasts) were washed three times with 1 M sorbitol, 25 mM EDTA pH 8.0 solution to remove nuclease contamination. Cells from either one cellophane-agar plate or one flask were collected in an Eppendorf tube and further processed according to one of the following methods:

1. Cells were suspended in 500 µl 50 mM EDTA, pH 8.0, 0.3% SDS solution. Then, 2 µl diethyl pyrocarbonate was added. The mixture was incubated at 65°C for 1O min and cooled on ice. After addition of 100 µl 5 M K-acetate the precipitated proteins were centrifuged in an Eppendorf centrifuge for 5 min. The nucleic acids were precipitated from the supernatant by 2 volumes of ethanol, collected by centrifugation and dissolved in 40.50 µl of 10 mM Tris pH 8.0, 10 mM NaCl, 1 mM EDTA. RNA was removed by adding pancreatic RNase to a final concentration of 25 µl/ml and incubating at 37°C for 1 hour.

2. Cells were suspended in 500 µl 50 mM Tris pH 8.0, 5 mM EDTA solution and 25 µl 20% SDS was added. After 10 min incubation at room temperature 125 µl 5 M NaCl was added. The lysate was incubated on ice for 1 hour then centrifuged for 5 min in an Eppendorf centrifuge. RNase was added to a final concentration of 25 µg/ml and the mixture was incubated at 37°C for 1 hour. Then it was extracted with 0.5 vol phenol, 0.5 vol chloroform and twice with an equal volume of chloroform. DNA was precipitated with 2 volumes of ethanol, collected by centrifugation and dissolved in 40-50 µl 10 mM Tris, pH 8.0, 10 mM NaCl;, 1mM EDTA.

Both protocols are suitable for the parallel preparation of DNA from several independent cultures. The purification can be carried out throughout in Eppendorf tubes of volumes 1.5 or 2 ml. The DNA provided by these methods is pure enough to be digested by restriction endonucleases and to be used in Southern type experiments. 20-30 µg DNA can be obtained from one cellophane-agar plate or from 100 ml liquid overnight culture. This amount of DNA is sufficient for several digestions. (Supported by the Hungarian Academy of Sciences.) Institute of Biology, University Medical School, H-4012 Oebrecen, Hungary.