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Preparing Neurospora DNA: some improvements.

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Abstract

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Our need to prepare DNA suitable for restriction enzyme analysis from rather large numbers of different strains led us to develop an easy method for making DNA (Metzenberg and Baisch 1981 Neurospora Newsl. 28: 20-21). Since then, we have made some small modifications that result in significantly improved yield and quality of product without increase in effort. The changes are as follows:

(1) "Extraction buffer" has been modified by substituting triethylamnonium ethylenediamine tetraacetate (TEA.EDTA) for lithium EDTA, and by adding a modest concentration of NaCl. The evolution of the procedure started with the sodium salt of EDTA, which comes out of ethanol as an intractable syrup. We then switched to the lithium salt, which does not "syrup out" even at high concentrations, but may crystallize out of ethanolic solutions, especially once the lab has been seeded. The triethylamnonium salt has given no trouble of any kind - except that we have come full circle and must add a small amount of sodium ion to insure precipitation of the DNA!

The TEA.EDTA is made as a 500 mM stock solution by suspending 73 g (250 mmoles) of the free acid of EDTA in 350 ml of water and adding, with continuous stirring, triethylamine base to give pH 8.0 8.2. (The base should be redistilled if colored impurities are evident.) In our hands, the volume of base required is 108.5 ml, but no doubt this will vary, depending on the water content of the triethylamine. The stock solution is then diluted to 500 ml. We have stored it frozen, though this may not be necessary.

The final concentrations in "extraction buffer", then, are: TEA.EDTA, 250 mM; Triton X-100, 0.5%; NaCl, 50 mM; and bacterial proteinase, 250 µg/ml.

(2) Extraction of the powder for 2-3 days at 33° or 37° with gentle agitation appears to give somewhat larger yields than overnight extraction without any agitation. This seems to be especially true of scaled-up preparations.

(3) All precipitations, either with ethanol or ethanolic perchlorate, are done with reagents and the preparations at room temperature. No incubation of the ethanolic suspensions, either at 4° or at room temperature,

is necessary to insure completeness of precipitation of DNA

(4) Rather than collect the precipitated DNA by centrifugation, which sediments any alcohol-insoluble impurities along with the DNA, we immediately collect the fibrous mass of DNA with an arrowhead-tipped plastic cocktail toothpick such as are available at supermarkets or liquor stores. (The toothpicks are freed of possible traces of DNase by dipping them into IN NaOH and then into sterile water). Excess liquors are drained from the mass of the toothpick. The cloudy DNA-free suspension is discarded and the DNA is dissolved in the same tube in 0.8X the desired final volume of low-salt buffer at room temperature. This usually takes less than 30 minutes; then 0.2 volumes of 5X high salt buffer is added if the procedure is to be stopped at that point for more than an hour, or the final preparation is to be stored. - - Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706.