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Abstract

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Lithium acetate has been used with success in preparing Neurospora conidia for transformation (Dhawale, Paietta and Marzluf, 1984 Curr. Genet. 8: 77-79). The lithium acetate procedure provides a means for rapid and efficient transformation of Neurospora with, frequencies of stable transformants comparable to or better than those obtained with other protocols. In addition, we have found that impure plasmid DNA such as that isolated by rapid preparation techniques (i.e, rapid alkaline extractions) can be used directly for transformation with this procedure. This note provides further information on the results obtained with plasmid minimpreps.

The lithium acetate procedure was carried out as described (Dhawale, Paietta and Marzluf, 1984 Curr. Genet. 8: 77-79). The technique involves treatment of germinated conidia with (1) 0.1 M lithium acetate, (2) plasmid DNA in 0.1 M lithium acetate, and (3) 40% PEG in 0.1 M lithium acetate. The final step is a heat shock prior to plating on selective medium. Gentle shaking is used throughout the procedure and most steps are carried out in Corning plastic tubes (#25311).

Data for two plasmid isolation techniques, the rapid alkaline extraction of Birnboim and Doly (1979 Nucleic Acids Res. 7: 1513-1523) and the rapid boiling technique of Holmes and Quigley (1981 Anal. Biochem. 114: 193-197), are presented here. Plasmid DNA of pVK57 and pVK88 (Alton, et al., 1978 Gene 4: 241-259) containing the qa-2^{+} gene was prepared from 30 ml chloramphenicol-amplified E. coli cultures by these techniques. The plasmid DNA preparations were treated with RNase and ethanol-precipitated in the presence of 2.5 M ammonium acetate prior to being used for transformation of the Neurospora strain qa-2;aro-9;inl.

TABLE I

Transformation of Neurospora with miniprep DNA samples

plasmid	size	no. of stable qa-2+ transformants[a,b] alkaline lysis method	boiling method
pVK57	7.t kb	34	19
pVK88	11.1 kb	13	6

[a] 20 µg of plasmid DNA was used.

[b] 5×10^7 conidia were used for each transforamtion and plated at a density of 1×10^6/plate.

Neurospora transformants were observed with both types of plasmid DNA minipreps (Table I). The alkaline extraction preparations proved to be the most effective for transformation. However, in all cases the number of transformants obtained was substantially lower (one-fifth to one-twentieth) than the number that would be obtained with the same amount of CsCl gradient purified DNA. Relatively impure plasmid DNA may, therefore, be useful only in cases where a high frequency of transformation is not required. Such uses could include isolation of minipreps of plasmid DNA from a set of Bacterial clones to check each for transformation of a Neurospora mutant as a step in gene cloning or during subcloning procedures. (Supported by grant GM-23367 from the National Institutes of Health and grant PCM-8013042 from the National Science Foundation). - - - Department of Biochemistry, Ohio State University, Columbus, OH 43210.