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Abstract

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A rapid and efficient approach for *Neurospora crassa* transformation using low melting point agarose purified DNA.

Sib selection, which takes advantage of a highly efficient transformation system (Akins, R.A. and A.M. Lambowitz. 1985 Mol. Cell. Biol. 5:2272-2278; Vollmer, S.J. and C. Yanofsky 1986 PNAS USA 83:4869-4873), has been an efficient cloning method for *Neurospora crassa*. Normally, CsCl gradient purified DNA is required to achieve high transformation efficiency. We have found that miniprep DNA prepared by the boiling method or by the alkaline lysis method (Maniatis, T. et al. 1982 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory pp. 366-369) can be used to transform Neurospora at high efficiencies if it is purified through LMP (low melting point) agarose. DNA from restriction and modification reactions can also be purified in this way. In our hands, the transformation efficiency of cosmid DNA prepared in this manner approaches that of CsCl gradient purified cosmid DNA (Table 1). Transformation efficiency shows strain dependence (see also Clements, A. and G.A. Marzluf. 1985 Neurospora Newsletter 32:6), and also varies among individual preparations of the same DNA clone. It is possible that some LMP agarose preparations will not work as well as others; the only one we have used in SeaPlaque LMP agarose from FMC Bioproducts, Rockland, ME.

We have successfully used this method for the sib selection and subcloning of *arg-13*, cloning of *os-1*, and other purposes. For sib selection, cosmid clones in 96 well microtiter dishes are replicated into ampicillin/LB plates (liquid cultures have not been used in order to minimize the effects of differential growth rates of individual clones). Bacterial colonies arising from overnight growth at 37°C are resuspended in 15 ml sterile HOH, collected by centrifugation, and processed by the alkaline lysis method. After ethanol precipitation, DNA is resuspended in TE (10 mM Tris-HCl pH 8.0, 1 mM Na2EDTA), mixed with equal volume of 5 M LiCl, microfuged after 10 minutes ice incubation (Pelham, H. 1985 Trends Genet. 1:6) and then precipitated once more with ethanol (Vollmer and Yanofsky op cit.). DNA is resuspended in 40 ul HOH, and 20 ul is loaded on a 0.5% LMP gel with 0.5 X TBE (Maniatis et al. op cit., p. 156) as the running buffer. Electrophoresis is at 20-60 volts for 6 hours to overnight, and we usually load only alternate wells to minimize cross contamination. Cosmid DNA will appear as a smear from the point of loading; the whole band is cut out, avoiding any excess liquid and agarose in order to maximize DNA concentration. We usually attain a final yield of 4 to 10 ug DNA in 100 to 400 ul LMP agarose. The volume of the cosmid DNA can be minimized by electrophoresing it only a short distance; this does not appear to affect the purity significantly.

We basically follow the Vollmer procedure for transformation. In sib selection, we normally use 20-60 ul (0.5-4 ug) pool DNA in LMP agarose, melted at 65°C, diluted to 160 ul with warm HOH, and then add 10 ul spermidine 3HCl (50 mM) and 25 ul heparin (5 mg/ml in 1 M sorbitol/50 mM Tris Cl, pH 8.0/50 mM CaCl2) and mix well. Add 240 ul 1M sorbitol/50 mMTris Cl, pH 8.0/50 mM CaCl2 and 60 ul 40% w/v PEG 3350/50 mM Tris Cl, pH 8.0/50 mM Cacl2 and mix well, then add 2 x 10^7 spheroplasts (200 ul, 10-25% viability, 99.9% spheroplasting) and mix. The mixture is transferred to ice water (to achieve rapid cooling) for 30 minutes, then moved to room temperature. 5 ml 40% w/v PEG 3350/50 mM Tris Cl, pH 8.0/50 mM CaCl2 are added. After 20 minutes at room temperature, 25 ml regeneration agar are added, mixed and the mixture plated onto bottom agar containing 0.5 ug/ml benomyl in 150 x 15 mm petri dishes. Normally, transformants will grow up after two days incubation at 30°C. It is critical to keep IMP agarose liquid before adding the spheroplasts. If LMP agarose resolidifies, reheat it at 65°C for 5 minutes. Temperature of the transformation mixture should not exceed 40°C just before the addition of spheroplasts. Transformation can be scaled down if fewer transformants are needed.

TABLE 1. Numbers of stable transformants (± one standard deviation) obtained when 2 x 10^7 spheroplasts were transformed as described in the text with 1 ug of cosmid DNA prepared wither by CsCl gradient or by alkaline lysis followed by purification through LMP agarose. Numbers reported are pooled from many transformations. The variability among LMP agarose preparations is partially due to the use of different sources of DNA (different cosmid pools). Inaccuracies in the estimation of DNA concentration also contributes to variability, since estimates based on fluorescence in agarose gels cannot be extremely precise

	Sources of cosmid DNA	
Spheroplasts	LMP agarose	CsCl gradient
bd al-2 arg-13 a	1670 ± 776	1940 ± 394
bd A	1450 ± 619	1800 ± 307

Purification of miniprep DNA through LMP agarose has several advantages: (1) it is rapid, with no need of an ultracentrifuge; (2) DNA so prepared efficiently transforms Neurospora; (3) DNA so prepared can be used for a number of other purposes without further purification: it can be used for restriction and modification reactions, bacterial transformations (Struhl, K. 1985 Biotechniques 3:452), and radioactive labelling (Feinberg, A.P. and B. Vogelstein. 1984 Anal. Biochem. 137:266-267).

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