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

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

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9. Add 10 ul NaOAc and top off the eppendorf tube with EtOH. Invert gently several times. Nice DNA "ropes" appear again.

10. Microcentrifuge as above 30 seconds to 2 minutes to pellet the DNA. Pour off the supernatant and rinse the pellet with 70% EtOH. Invert to drain 1 minute.

11. Dry the tubes in a vacuum oven at 50° C for 15 minutes at most.

12. Resuspend the DNA pellet in 100 ul of TE and store the tubes at -20° C. 5 to 10 ul is plenty for a digest. Treat with RNase as called for by the method of Zolan and Pukkila. -- -- Department of Botany, UC Berkeley, Berkeley, CA 94720. # Current address: Department of Plant Pathology, Cornell University, 334 Plant Science Bldg. Ithaca, NY 14853-5908.

Liu, Q. and J. C. Dunlap 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 4 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 LMP 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 × 10^7 spheroplasts were transformed as described in the text with 1 ug of cosmid DNA prepared with either 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.

<table>
<thead>
<tr>
<th>Sources of cosmid DNA</th>
<th>Spheroplasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMP agarose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bd al-2 arg-13 a</td>
</tr>
<tr>
<td></td>
<td>bd A</td>
</tr>
<tr>
<td>CsCl gradient</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1800 ± 307</td>
</tr>
</tbody>
</table>

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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Mattern, I.E., P.J. Punt and

A vector of Aspergillus
transformation conferring
phleomycin resistance.

expressed A. nidulans gpd gene, and the

Recently, transformation of Aspergillus species with vector pAN7-1, conferring resistance to hygromycin B was reported (Punt et al. 1987 Gene 56:117-124). Here we describe a transformation vector (pAN8-1, Fig. 1) containing the Streptococcus hindustandus phleomycin resistance gene (obtained from G. Tiraby, Toulouse, France) flanked by the promoter region of the highly expressed A. nidulans trpC gene.

Transformation of A. nidulans and A. niger was achieved with this vector at frequencies of 1 to 20 transformants per ug pAN8-1 DNA. These frequencies are similar to those found for transformation with pAN7-1. Transformants could be selected at low concentrations of phleomycin (5-10 ug/ml for A. niger, 10-20 ug/ml for A. nidulans).

A. oryzae, which cannot be transformed with pAN7-1 because of its innate insensitivity to hygromycin B, is inhibited in its growth at 50-100 ug/ml phleomycin. Phleomycin resistant transformants were obtained by cotransformation of an A. oryzae pyrG mutant with pAB4-1 (containing the A. niger pyrG gene) and pAN8-1 (Mattern et al. 1987, MGG 210:460-461). Experiments are in progress to achieve direct selection of phleomycin resistant transformants of A. oryzae.

Figure 1. Vector pAN8.1. A 0.4 kb NcoI-StuI fragment from pUT701 (G. Tiraby, unpublished) containing the coding region of the S. hindustandus phleomycin resistance gene was ligated into pAN52-3, which was cut with HindIII, treated with T4 polymerase and subsequently cut with NcoI. Vector pAN52-3 is a derivative of pAN52 (Punt et al. 1987 Gene 56:117-124) in which the unique BamHI site was converted into a HindIII site by site directed mutagenesis.