Colony hybridization has proven to be useful procedure for efficiently screening bacterial clones by RNA-DNA or DNA-DNA hybridization (Grunstein and Hogness, 1975 Proc. Natl. Acad. Sci. USA 72: 3961-3965). Such a technique would also be useful in Neurospora for the detection of specific DNA sequences, as in the case of integrated vector sequences in a transformant. The following protocol, which is a modification of the procedure used in yeast (Hinnen, et al., 1978 Proc. Natl. Acad. Sci. USA 75: 1929-1933), allows for colony hybridization to be carried out in Neurospora.

S & S BAB5 nitrocellulose filters were autoclaved and then dipped into Fries medium containing 1.8% sorbose. The moistened filters were placed on agar plates of the same medium that had been overlaid with 1-2 ml of liquid medium. Droplets of conidial suspension from each strain to be tested were then spotted on the filters. The inoculated filters were incubated for 24 h at 30° C. The filters were then air-dried briefly and placed, in the following sequence, on Whatman 3 MM paper saturated with: (1) M sorbitol, 40 mM EDTA (pH 8.0), 50 mM DTT, 15 min, 30° C; (2) 1 M sorbitol, 40 mM EDTA (pH 8.0), 10% glycosulase (Endo Labs., 90,000 units/ml), 3-4 h, at 37° C; (3) 0.5 M NaOH, 10x SSC, 8 min; (4) 0.5 M Tris-HCl (pH 7.4), 10x SSC, 4 min; and (5) 0.5 M Tris-HCl (pH 7.4), 10x SSC, 4 min. The filters were subsequently transferred to a S & S filtration block (#MV082) and, with suction applied, 200 ml of 3x SSC and 100 ml of chloroform were poured through the filter. Following air-drying the filters were baked in a vacuum oven at 80° C for 2 h.

The baked filters were soaked in 3x SSC for 15 min followed by 2 h in 3x SSC, 10x Denhardt’s sol., 0.1% SDS at 65° C. Prehybridization was in 3x SSC, 10x Denhardt’s sol., 0.1% SDS and 1 mg/ml salmon sperm DNA for 12 h at 65° C. Hybridization was carried out for 24 h at 65° C followed by washes in 3x SSC, 2x SSC, and 1x SSC. For autoradiography Kodak XAR-5 film and DuPont Cronex intensifying screens were used. A light background was usually present in the autoradiographs on all of the colonies but did not interfere with scoring a positive response to the probe.

Figure 1 shows a colony hybridization screen of some homokaryotic Neurospora qa-2+ transformants. The probe was 32P-labeled pBR322. A single colony has given a positive response indicating that at least part of the vector (pBR322) has integrated along with the qa-2+ gene in this transformant.

Lithium acetate has been used with success in preparing Neurospora conidia for transformation (Dhawale, Paietta and Marzluf, 1984Curr. Genet. 8: 77-79). The lithium acetate procedure provides a means for rapid and efficient transformation of Neurospora with plasmid DNA. 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.

The lithium acetate procedure was carried out as described (Dhawale, Paietta and Marzluf, 1984Curr. 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 1

Transformation of Neurospora with miniprep DNA samples

<table>
<thead>
<tr>
<th>plasmid</th>
<th>size</th>
<th>alkaline lysis method</th>
<th>boiling method</th>
</tr>
</thead>
<tbody>
<tr>
<td>pVK57</td>
<td>7.7 kb</td>
<td>34</td>
<td>19</td>
</tr>
<tr>
<td>pVK88</td>
<td>11.1 kb</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

*20 µg of plasmid DNA was used.

*5 x 10⁷ conidia were used for each transformation and plated at a density of 1 x 10⁶/plate.

Neurospora transformants were observed with both types of plasmid DNA minipreps (Table 1). 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.)

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**Perkins, D. D.**

Griffiths and DeLange (1978 Genetics 88: 239-254) and Griffiths (1982 Can. J. Genet. Cytol. 24: 167-176) have obtained numerous null mutations of the mating-type alleles in Neurospora crassa. Typically the mutant strains have lost simultaneously both mating ability and the vegetative incompatibility (heterokaryon incompatibility) that is also a normal property of the mating-type alleles. Loss of the mating reactions cannot be restored by complementation. Unlike normal A and a strains, the mating-type null mutants are capable of forming heterokaryons both with A and a partners (pφOI). The strains are alike at other heterokaryon-incompatibility loci. When such a heterokaryon is used as parent in a cross, only genes from the component having an active mating-type allele are transmitted. The component with the inactive mating-type allele does not contribute appreciably to the progeny.

We have found an inactive-mating-type strain to be ideal as a component of heterokaryons used to rescue or to normalize disadvantaged strains having recessive genotypes that render the strains lethal, weak, infertile, or unstable. Heterokaryons containing such a disadvantaged component together with the inactive-mating-type helper are phenotypically wild-type in morphlogy, nutrition, and growth rate. They are also fertile in crosses when used as either female or male parent.

I have employed as helper in heterokaryons a strain kindly provided by Dr. A. J. F. Griffiths -- a m³ ad-3B cyh-1 (alleles Nos. 1, 12-17-11A, H521(r); now deposited as FGSC #4564). (We have found the notation a m³ more convenient than a m³ used earlier.) Allele a m³ is one of three nonreverting a mutants reported in 1978. (Seemingly contradictory statements in this 1978 Genetics paper are due to four typographical errors: "mutant 1") should read instead "mutant 11" at line 15 on page 249, lines 7 and 19 on page 250, and in Table 2. -- Griffiths, personal communication.) ad-3B serves as forcing marker. cyh-1 can be ignored in the present context. The strain will be referred to as "a m³ helper" or simply "helper".

The a m³ helper is heterokaryon compatible with the Oak Ridge Laboratory wild types, which are het-C het-d het-b.