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Rapid miniprep of DNA from filamentous fungi

Routine screening of a large number of transformants by Southern or slot blot analyses requires rapid DNA isolation from small fungal cultures. We have developed a procedure which allows the easy preparation of DNA from 100 or more isolates per day. This technique has been used successfully with Aspergillus nidulans, A. niger, Penicillium chrysogenum and Neurospora crassa.

1. Inoculate 10 ml of medium with a loopful of conidia. Incubate for 16-30 h.

2. Harvest mycelium on Whatman #1 paper and rinse with distilled water. [We get 50-200 mg (wet weight) of mycelium.]

3. Put mycelium in a 12 X 75 m glass tube. (Note: while not necessary, the yield of DNA is much better if the mycelium is lyophilized and/or mechanically diced at this stage.)

4. Add 0.7 ml LETS buffer (0.1 M LiCl, 10 mM EDTA, 10 mM Tris pH 8.0 and 0.5% SDS) and then add glass beads (0.45 mm) to the top of the liquid.

5. Cover tubes with parafilm and vortex for 1-2 min. We use a multitube vortexer at top speed.

6. Add 1 ml of phenol:chloroform:isoamyl alcohol (25:24:1) to each tube, vortex (medium speed) for 20 sec.

7. Centrifuge at 3000 rpm in a clinical centrifuge for 5 min.

8. Transfer 500 ul of the aqueous phase to a clean microfuge tube, add 1 ml 100% ethanol and put on dry ice for 15 min.

9. Spin for 15 min in a microfuge at 4° C, remove supernatant and dry the pellet.

10. Resuspend the pellet in 40 ul TE (10 mM Tris, 1 mM EDTA pH 8.0).

The resultant DNA is quite dirty (colored solutions are not uncommon) so we have found that restriction digests are most successful when done in a volume of 400 ul. We use 10 ul of the miniprep with 20 U enzyme for 5 h (or overnight) and precipitate the DNA (with 1/10 volume 5 M ammonium acetate plus 2 vol. 100% ethanol) before running an agarose gel. The pellet is resuspended in loading buffer containing RNAse before loading onto a gel.

The DNA size is >23 kb and will digest cleanly in large volumes. The yield is approximately 1-5 ug of DNA from the prep using fresh mycelium and can be increased to 10-30 ug by lyophilization of the mycelium and/or mechanical dicing of the mycelium before adding the LETS and the glass beads. - - - Panlabs Research, 1550 N. 115th St, Seattle, WA 98133.