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Abstract
Fungal genetic studies require a rapid method of isolating DNA from a large number of samples for restriction enzyme analysis.
Complementation studies including mts(MN9), cpc-1 (CD86 and j5) and mts(MN1) were performed as already described for mts(MN1) (Koch and Bartheless, 1987). The mutants were recessive to their respective wild type alleles, but complementation of the amino acid analogue sensitive phenotype was not observed in heterocaryons carrying mutant alleles simultaneously. These findings suggest that cpc-1, mts(MN9) as well as mts(MN1) belong to the same complementation group. --- Institut für Angewandte Genetik, Universität Hannover, 3000 Hannover FRG. Supported by the Deutsche Forschungsgemeinschaft.

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A rapid, high yield mini-prep method
for isolation of total genomic DNA
from fungi.

Fungal genetic studies require a rapid method of isolating DNA from a large number of samples for restriction enzyme analysis. Previous methods we have used are limited by relatively low yield of 50 ug DNA/0.1g lyophilized mycelium (Zolan, M.E. and P.J. Pukkila 1986. Mol. Cell. Biol. 6:195-200) or tedious gel exclusion column chromatography (Biel, S.W. and F.W. Parrish 1986. Anal. Biochem. 154:21-25). In addition, these two methods yielded no readily digestable DNA from Phytophthora cinnamomi.

The following method facilitates rapid isolation of large quantities of easily digested total, genomic DNA from several species of Phytophthora, including P. cinnamomi, and several species of Boletus, Chroogomphus vinicolor, Gomphidius glutinosus, Leccinum manzanitae, Magnaporthe grisea, Neurospora crassa, N. tetrasperma, Omphalotus olivascens, and Talaromyces flavus. Yield was increased to 200 ug DNA/0.1 g lyophilized mycelium and isolation of DNA from two to three times as many samples can be achieved using this rapid method (current record is 64 isolates in one day versus 24 using previous methods). DNA has been successfully cut with all restriction enzymes tried to date.

Solutions needed:
1. Lysis buffer: 50 mM Tris-HCl
   50 mM EDTA
   3% SDS
   1% 2-mercaptoethanol (add just before use)
2. Chloroform:phenol (1:1)
3. SEVAG (chloroform:isoamyl alcohol, 24:1)
4. 3 M NaOAc (pH 8.0)
5. Isopropanol
6. Ethanol (100%, -20°C)

Protocol:
1. Fill a 1.5 ml eppendorf microcentrifuge tube 2/3 to the joint with ground lyophilized mycelium (60-100 mg dry, or 0.5-1.0 g wet, ground in liquid nitrogen).
2. Add 750 ul of lysis buffer, stir with a dissecting needle and/or vortex so the mixture is homogenous. Incubate at 65°C, 1 hr.
3. Add 700 ul of chloroform:phenol; 1:1 and vortex briefly. Microcentrifuge at 12,000 x g for 10 minutes or until the aqueous (top) phase is clear.
4. Remove 600-650 ul aqueous phase to a new tube -- be careful not to take any cellular debris from the interface. Don't get greedy!
5. Add 700 ul of SEVAG, vortex briefly. Be careful, as the caps are loosened by chloroform. Microcentrifuge as above for 5 minutes.
6. Remove the aqueous phase to a new tube (approx. 550-600 ul). Add 20 ul of 3 M NaOAc. Top off the eppendorf tube with isopropanol. Invert gently several times. You should see DNA "ropes" precipitate.
7. Microcentrifuge as above for 30 seconds to pellet the DNA. Pour off the supernatant. Invert the tubes for 1 minute to drain.
8. Add 300 ul TE and place in a heat block at 65° C for 10-15 minutes. Finger vortex to resuspend the pellets.
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 described above.

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 agarose 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 mM Tris 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.