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S. B. Lee

M. G. Milgroom

J. W. Taylor

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

Abstract

Fungal genetic studies require a rapid method of isolating DNA from a large number of samples for restriction enzyme analysis.

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Lee, S.B., M.G. Milgroom # and J.W. Taylor

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 after the digest 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.