How to amplify DNA from macroconidia

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Background

To test the genotype of a strain or to amplify a region of genomic DNA for cloning or sequencing it is not always necessary to first extract and purify genomic DNA from other cellular constituents. The region of interest can be amplified directly from a suspension of asexual spores. Neurospora produces three distinct types of spores: ascospores, macroconidia and microconidia. Ascospores are the product of a sexual cross between strains of opposite mating type[1]. Macroconidia and microconidia are asexual spores. Macroconidia contain between 1-5 nuclei and are produced in abundance on most media. Microconidia are much less abundant, smaller than macroconidia and contain most often one, but occasionally two nuclei[2].

Procedure

For Neurospora template DNA harvest macroconidia (5-10 days old) with a loop or sterile toothpick from slants. Transfer to 100 µl sterile dH_2O and vortex. Dilute 1µl in 999 µl dH_2O and measure the OD_{530} reading to estimate the number of spores using the following formula:

OD_{530} x dilution factor x 10^6 = conidia/ml

*For amplification use 2 x 10^4 spores/ 50 µl reaction. Incubate the spore suspension at 30 °C for 75 minutes then freeze in liquid nitrogen.

dH_2O	31.75 µl
10 x PCR buffer	5µl
macroconidia * (10^6 spores/ ml)	2µl
10 mM dNTP mix	1 µl
forward primer 50 pmol/ul	5 µl
reverse primer 50 pmol/ul	5 µl
taq polynmerase (5 units/µl)	0.25 µl
Total volume	50 µl

The first denaturation step should be at least 5 minutes at 95 °C. Subsequently denature for 30 to 60 seconds at 95 °C. Carry out 40 cycles of denaturation, annealing of primers, and extension.

Henderson *et al*.[3] have reported a slightly different method from the one described above. A loop full of conidia is suspended in 100 µl Tris-EDTA (pH 8.0), placed in a boiling water bath for 5 minutes then transferred to ice. The sample is then placed in a

microcentrifuge and spun at 13 K rpm for 5 minutes to pellet the cell debris. 5 µl of the supernatant is used per 50 µl PCR. They note that successful amplification of the genomic DNA is dependant on the polymerase used. In their hands Red Hot DNA polymerase from ABgene works well.

References

1. Shear, C. L. and Dodge, B. O. (1927) Life Histories and heterothallism of the red bread mold fungi of the *Monlia sitipholoia* group. J. Agric. Res. 34, 1019-1042.
2. Dodge, B. O. (1932) The non-sexual and sexual functions of microconidia of neurospora. Bull. Torrey Botan. Club. 59, 347-360.
3.Henderson S. T., Eariss G. A. and D. E. A. Catcheside (2005) Reliable PCR amplification from *Neurospora crassa* genomic DNA obtained from conidia Fungal Genet. Newsl. 52: 24.