A practical method for the preparation of total DNA from filamentous fungi

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Most methods of DNA preparation from fungi are time-consuming due to the need to first make protoplasts, expensive for chemicals such as cesium chloride, or suitable only for small scale preparations. We have developed a simple method for total DNA preparation, yielding a product of quality suitable for restriction digestion and library construction.

To extract DNA, 0.5 to 2.0 g of fresh or frozen mycelium is ground in liquid nitrogen. The mycelial powder is divided between two Sorvall tubes each containing 15 ml of cold spermidine-SDS buffer (4 mM spermidine, 10 mM EDTA, 0.1 M NaCl, 0.5% SDS, 10 mM ß-mercaptoethanol, 40 mM Tris-HCl pH 8.0), and thoroughly shaken. The mixture is then immediately extracted with 1 vol double distilled phenol. After a second phenol extraction, the aqueous phase is extracted with 1 vol chloroform. To the resulting aqueous phase is added 10% of that volume of 3M sodium acetate pH 5.5. DNA is then precipitated by addition of 2 vols of cold absolute ethanol, and recovered either by spooling it out or by centrifugation at 10,000 x g for 10 minutes. After washing with 70% cold ethanol, the DNA is air-dried at room temperature, redissolved in TE buffer (20 mM Tris-HCl pH 7.5, 0.1 mM EDTA) to a DNA concentration circa 0.5 mg/ml and stored at -20°C. Purity and average fragment size are checked by electrophoresis.

The method is highly reproducible, and results over 70% recovery of good quality DNA (A_{260}/A_{280} circa 1.85). With optimal grinding and nuclease inhibition, the DNA is of high molecular weight (>23 kb), with minimal smear on electrophoresis. The method has been used for *Neurospora crassa, Aspergillus nidulans, Dactylium dendroides* and *Humicola grisea*. For all four species, the DNA prepared has been successfully restricted and religated. DNA from this method has been used in the construction of genomic libraries from *Humicola grisea* and *Dactylium dendroides*.